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# Goal 3: Biomarkers of Welfare

Darian Pollock, Giuliana Miguel-Pacheco, Herman Coceancigh, Yolande Seddon

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# Program objectives

1) To understand how early life management contributes to pig robustness, sociability and welfare outcomes in the growing pig

2) Identify if promotion of play, can increase physiological and psychological robustness

3) Identify and validate biological markers indicative of welfare states in swine

4) Understand the value of animal-based post-mortem indicators of welfare

# Goal 3 – Biomarkers of welfare

## The Challenge

- Increasing pressure for industries to respond to welfare concerns
  - Complex welfare questions being posed, challenging to answer.
- Criticism and bias's towards production system types

## The need:

- Objective measures that remove bias
- Longer-term measures – measures that could reflect on the animals overall welfare over their productive life, or a specific duration of time.
  - Understand housing system effects.
- Can be used in all systems

a) New research tools – help answer tough questions

b) Industry monitoring tools – sensitive, accurate monitoring of animal welfare – lead time indicators of change to reduce loss.

c) Genetic selection tools

# Goal 3 – Objectives and Progress

- **Objective** - Broad: Identify and validate a selection of promising biomarkers as they relate to welfare state in swine.

- **Areas of focus**

**1) Chronic measures:** Identify a robust method to evaluate welfare over a longer period of time

- Evaluate if an animal is affected by chronic stress, is coping or demonstrates stress resilience.
- Cortisol and DHEA in swine hair

**2) Automated monitoring of consumption behaviours**

- Can it provide a lead time indicator of change
- Allows linking welfare measures to productivity

# Progress: Automatic Feeders

- Sixteen feeders installed at the Prairie Swine Centre January 2020
  - Funded by: Canada Foundation for Innovation and University of Saskatchewan
- 8 x nursery feeders, 8 x grow/finish feeders

1st Piglet feeders in

- No issues with weaned piglets feeding from them at weaning

Increases research capabilities

- Precise & objective feeding data
- Link productivity, physiology, behaviour and welfare


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Automated filling system

Adjustable gates (width & height) access to feeder for 1 animal

**No more than 15 animals per feeder**

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# Feeding data

- Individual feeding information:
  a) Feed consumption per visit and day (kg)
  b) Time of feeding
  c) Number of feeding events per day
  d) Duration of time feeding per day

May 2020: Test run of system

June 2020: Experimental pigs fed on feeders

## In 2020

**Goal 3: Influence of rearing environment on cortisol and DHEA as measures of long term stress physiology in swine**

- Pigs tracked birth to slaughter – first full test run on feeders over seasons
  - Identify the seasonal challenges
- Systematic weekly welfare and health monitoring applied
- Captured tail biting outbreak

## In 2021 & 2022

Pigs studied under the research projects of

Goal 1 and 2 will be fed on feeders

- Consumption data
- Production efficiency
  - Layer measures – to model against welfare measures

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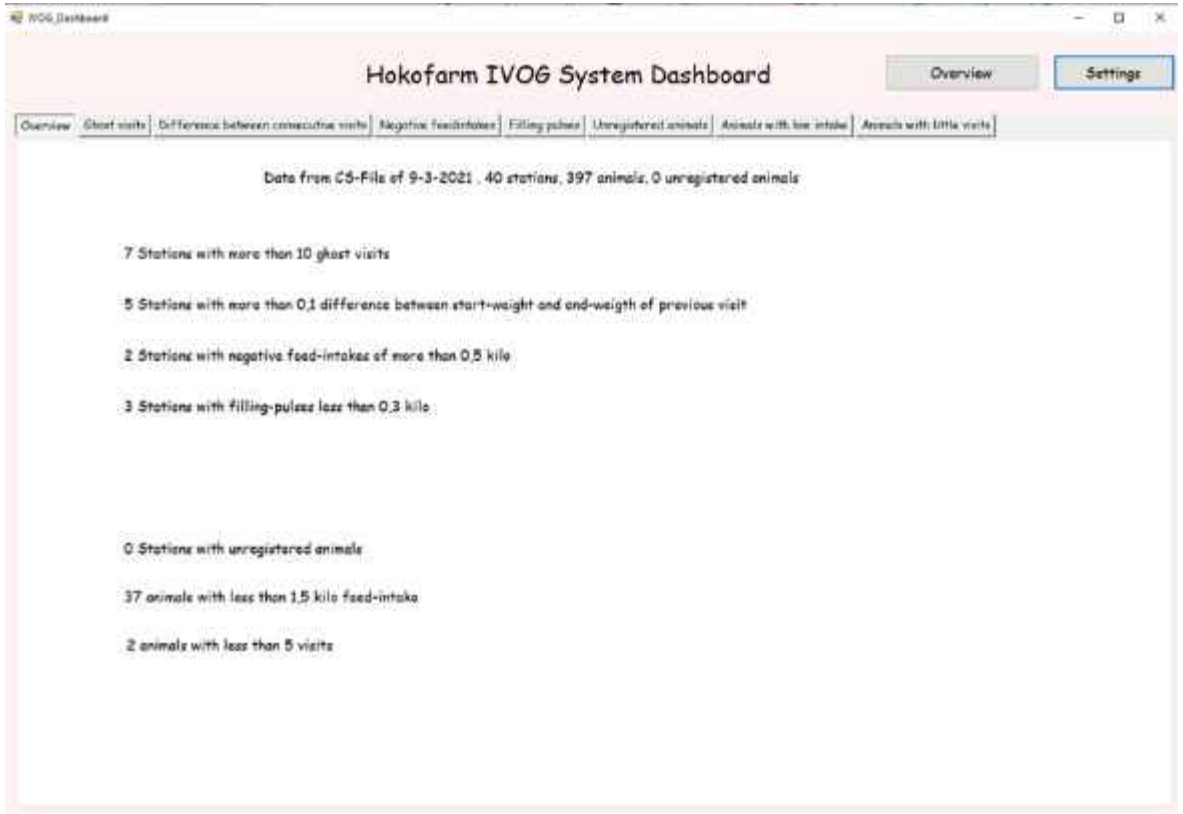
# Automated Feeders: Data management




- 2020 – Developed a system for efficient data handling
  - Linux script for data handling
  - Collate feeding data from all pens automatically
- 2021:
  - Data collation refining and maintenance for studies - Herman Coceancigh
  - Data statistics for first Goal 3 experiment – Darian Pollock
  - Hokofarm IVOG – Testing new software

# Biomarkers of Welfare: Chronic measures

- Lack of objective biomarkers to inform on long-term stress in swine.

Biomarkers of chronic stress could:

- Inform on animal welfare in chronic conditions
  - Confinement housing, interventions

- Evaluate stress coping attempts in animals
- Evaluate stress responsiveness in animals
  - Markers to assist in genetic selection.

# Cortisol and Dehydroepiandrosterone (DHEA)

- Hormones released from the hypothalamic-pituitary-adrenal axis
  a) Higher levels of cortisol suggestive of increased stress (Casal et al., 2016; Morgan et al., 2019).
  b) Higher levels of DHEA suggestive of increased physical and mental well-being
- Blunted circadian rhythms suggested to relate to chronic stress (Munsterhjelm et al. 2010).

- Cortisol and DHEA identified as having largely opposing functions
  - Their ratio is suggested to be a superior measure (Kamin and Kertes, 2016).
  - Higher ratio suggestive of increased stress and chronic health issues.

Casal, N. 2016. Animal: 1-9. Morgan, L., 2019. Nature sci rep. 9: 16093. Munsterhjelm, C., et al., 2010. Orta, O.R., et al., 2020. Psychoneuroendocrinology. 112:104515. Wolkowitz, O.M., et al., 201. Psychiatry. 2(3): 115-143.

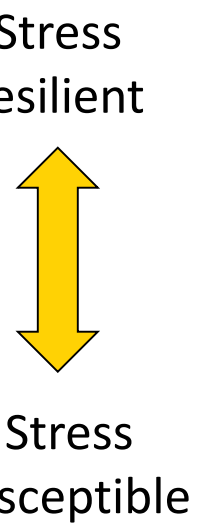
# Cortisol/DHEA ratio: Area of investigation

- Can we identify pigs that are stress susceptible vs resilient this ratio?
- Low cortisol/DHEA = more stress resilient.

Hair cortisol and DHEA concentration, hair cortisol/DHEA ratio, and interpretation (low/high ratio) of 19-week finisher pigs (n=8) raised under the same housing conditions*.

| Pig # | Sex | Cortisol (pg/mg) | DHEA (pg/mg) | Ratio (Cortisol/DHEA) | Low |
|---|---|---|---|---|---|
| 1 | F | 19.05 | 74.95 | 0.25 | |
| 2 | M | 13.22 | 33.54 | 0.39 | |
| 3 | M | 15.01 | 35.22 | 0.43 | |
| 4 | M | 19.72 | 42.24 | 0.47 | |
| 5 | F | 18.35 | 31.56 | 0.58 | |
| 6 | M | 14.75 | 22.30 | 0.66 | |
| 7 | F | 22.96 | 33.19 | 0.69 | |
| 8 | F | 16.75 | 17.30 | 0.97 | High |

* Pigs selected from two pens within the same batch.




Pollock et al. 2020, unpublished – NSERC IRC funded work

# Goal 3 – Biomarkers: Cortisol & DHEA

**Exp. 1: 2019 – 2020:** Evaluate how wash solvent (used to clean hair) & contamination level (fecal/urine)

- Influence analyte levels
- Validate a commercially available DHEA Elisa kit (Salimetrics, CA, USA).
- **Status: Complete, methodology publication submitted to journal, *Animals*,**
  - ***Special journal issue exploring physiology of chronic welfare measures***

- Methods: Hair sampled from 8 x growing pigs (900mg/pig)
  - Divided into groups of 100g and tested for
  - Washing with: Isopropanol vs methanol
    - Wash solvent influences analyte extraction in different species
      - Influence of hair type and lipids
  - Contamination: 25% and 75% of hair shaft for 48 hrs with feces and urine mix obtain from pig pen.
    - Number of washes required to remove contamination vs remove analyte
  - Validate assays for DHEA simultaneously.

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# Exp. 1 Conclusions and Value

- Results suggest changes to swine cortisol and DHEA hair protocol.

- Recommendations:
  - Do no use contaminated hair.
  - Wash visibly clean hair with three, three-minute methanol washes and visibly contaminated hair with five three-minute methanol washes.

- Value: More reliable measurement of cortisol and DHEA in swine hair

# Goal 3 – Biomarkers: Cortisol & DHEA

**Exp. 2: 2020 – 2021:** Evaluate the influence of rearing environment on DHEA and cortisol as measures of longer term stress physiology in swine

Objectives:

i) Establish system and pig age reference values;

ii) a) Understand how the circadian rhythm development of hormone levels and

b) Innate behavioural stress response

influences concentration of these hormones accumulating in hair

iii) Determine whether rearing system influences concentrations of DHEA, cortisol and their ratio in saliva and hair

iv) Determine whether concentrations of hair cortisol, DHEA and the ratio relate to measures of welfare

- This is necessary work to ensure correct interpretation of results in randomly sampled pigs

**Status:** Data collection complete, statistical analysis in progress.

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# Methods

- 16 litters reared birth – slaughter:
- 50% received straw from birth, half raised in standard environment.
- Hair shaved each growth stage
- Saliva taken over 12 hours at 12 and 20 weeks
  - Track circadian rhythm development
- Productivity taken each growth stage
  - Weights/stage: in and out
  - Individual pig feed intake: nursery and grow/finish
- Behaviour and health measures taken at repeated points from birth to slaughter.

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# Results: First look

**Hair hormone values (N = 64/stage): Mean and standard deviation**

| | Cortisol (pg/mg) | | DHEA (pg/mg) | | Cortisol:DHEA ratio | |
|---|---|---|---|---|---|---|
| | 12 weeks | 20 weeks | 12 weeks | 20 weeks | 12 weeks | 20 weeks |
| Straw | 11.5 ± 5.00 | 9.30 ± 4.76 | 28.4 ± 15.3 | 27.7 ± 16.9 | 0.55 ± 0.62 | 0.43 ± 0.27 |
| Barren | 13.2 ± 4.34 | 9.27 ± 3.70 | 27.5 ± 14.8 | 26.0 ± 16.6 | 0.61 ± 0.27 | 0.76 ± 1.49 |

**Analysis in progress:**

1. Saliva hormone concentrations, and their circadian rhythm
2. Feeding patterns, average daily gain
3. Behaviour budgets Skin lesions

Preliminary statistics to be completed by June 2021

Complete results by August 2021

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# Industry Value

- The hair cortisol:DHEA ratio shows promise as a relatively non-invasive, and objective biomarker of chronic stress in grower pigs.
- Exp. 2: Identifies if system specific effects need to be taken into consideration
- How individual pig characteristics relate to hair hormone levels
- Align measures to behavioural indicators of welfare
- Feeding data: Individual HPA activity to productivity

# Future studies: Exp. 3 and 4

**Experiment 3: Summer 2021**

- To determine if early life manipulations influences the concentrations of hair cortisol and DHEA, in addition to their ratio, and how this relates to measures of welfare, productivity and resilience
- Results expected in 2022

**Experiment 4: Possible avenue….**

- To identify the ability of hair cortisol, DHEA, and their ratio to inform on pig welfare on commercial farms.
- Can the measure be predictive of welfare status and meat quality?
- Data collection could align to Goal 4 data collection
- Results could be delivered in 2022